Nasal, axillary, and perineal carriage of *Staphylococcus aureus* among women: Identification of strains producing epidermolytic toxin

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Abstract

Following two outbreaks of staphylococcal scalded skin syndrome in a maternity unit, 500 pregnant women attending an antenatal clinic were screened for carriage of epidermolytic toxin producing *Staphylococcus aureus*. Nasal, axillary, and perineal swabs were collected from women whose gestational ages ranged from 12-40 weeks. Isolates of *S aureus* were purified, phage typed, and tested for methicillin sensitivity and production of epidermolytic toxin. The results showed that 164 (33%) women carried *S aureus*; of these, 100 (61%) were from the nose and three (2%) from axillae, but 41 (25%) strains were isolated from the perineum alone. Screening for nasal carriage alone will therefore miss 25% of carriers. More than one strain of *S aureus* was identified in seven of 20 women with multiple site carriage. Three (2%) methicillin resistant strains were isolated during the survey, and five (3%) isolates produced epidermolytic toxin. Phage typing identified 63 (34%) strains as non-typable, but 50% of isolates typed either groups I, II or III, and a further 10% represented varying combinations of these and other phage groups.

These results provide baseline information on *S aureus* in the community, and identification of methicillin resistant and toxin producing strains shows a reservoir of outbreak potential which could become relevant on hospital admission of such a carrier.

Nasal carriage of *Staphylococcus aureus* in normal populations is about 35% in both sexes,¹ but this may vary according to age² and race.³ A carrier may be unaware of the potential pathogenicity of the bacteria that they harbour, both to themselves and others.⁴⁻⁶ Newborn babies are particularly at risk from *S aureus* carried by mothers or attendant medical staff because of an immature immune system and the increased prevalence of *S aureus* in hospitals.^{2,7-9} Nasal carriage of infants on discharge from hospital approached 100% in a staphylococcal epidemic but is more usually about 60%.⁸ Colonisation or infection of the newborn is the principal route

by which the mother may also become infected, leading to the development of a breast abscess.¹⁰

S aureus of certain phage groups tend to be associated with particular skin diseases.¹¹ A rare neonatal disease called the staphylococcal scalded skin syndrome and the related milder, but more common form, pemphigus neonatorum, are generally caused by strains belonging to phage group II.^{12,13} Both these conditions fall within a continuous spectrum which ranges from localised bullous impetigo to a generalised epidermolysis of the skin resembling widespread first degree burns.¹⁴ The causative strain of *S aureus* colonising sites such as nose or umbilical stump in neonates¹⁵ produces epidermolytic toxins which are absorbed and eventually lead to separation of cells within the stratum granulosum of the epidermis.¹⁶ Toxin producing strains are identified by wrinkling or peeling of the skin (Nikolsky sign) in neonatal or hairless mice following subcutaneous inoculation of suspected isolates.^{13,17}

At least two epidemics of staphylococcal scalded skin syndrome have occurred in a London hospital maternity unit in consecutive years, the first affecting 12 babies,¹⁸ the second more than 80 neonates.¹⁹ Two sporadic cases and a mini outbreak affecting four babies and an adult have been identified more recently, all strains typed group II and produced a positive Nikolsky sign in the mouse bioassay.

The common appearance of this otherwise rare condition in our maternity unit prompted us to question the incidence of epidermolytic toxin producing staphylococci in the community. Epidemiological studies reporting the prevalence of toxin producing strains have generally examined isolates from clinical sources such as hospital patients or patients with superficial skin lesions²⁰⁻²³ but not from normal subjects. Immunological studies show that more than 50% of those over 10 years of age possess antitoxin and 80% of cord blood specimens contain epidermolytic toxin antibody,²⁴ which suggests that toxigenic strains certainly exist outside hospital but that the true prevalence remains obscure.

Similarly, the prevalence of different phage groups in the community is not well established. Groups I and II strains are said to colonise about 30 and 25%, respectively, of normal nasal carriers, while group III strains account for only 15% of carriers.¹ Phage group II staphylococci accounted for 8.2% of

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2969 isolates sent to the Division of Hospital Infection (CPHL) over a period of three months in 1986. Forty one (7%) of 584 nasal isolates sent during 1987–1988 were group II, but these were also from all sources, most being long term hospital inpatients (Marples RR, personal communication).

It was thus relevant to carry out a survey on a group of pregnant women living outside the hospital environment by examining nasal, axillary, and perineal sites for staphylococcal carriage. The results would contribute towards present knowledge of carrier rates in the general population because no difference in carriage is attributable to sex.^{25–28} Further useful epidemiological information would be obtained from an at risk group on carriage site, prevalence of antibiotic resistance, and different phage groups.

Methods

Five hundred women attending routine antenatal outpatient clinics were chosen at random to donate nasal, axillary, and perineal swabs. Gestational ages ranged from 12 weeks (booking) to 40 weeks (term) and permission was obtained from the mothers before swabbing took place. Three consultant clinics were visited weekly on a rotational basis over a period of three months; timing of visits was varied to avoid subject duplication and also to minimise the risk of collecting one strain of *S aureus* transferred between a social group. These women were healthy, aged 17–42 years, from all social classes, and had no medical histories of note. They did not work in hospitals or have any antenatal problems necessitating previous hospital admission or outpatient appointments other than the normal routine antenatal checkups. Brief questioning about partners' occupation and place of work helped to confirm eligibility for the survey, as subjects with indirect association with the hospital environment were excluded.

SWABBING PROCEDURE

Standard swabs were taken from three sites of potential carriage. It was regarded as sufficient to sample no more than the first centimetre of nasal epithelium when swabbing the nose,¹ and perineal swabs were taken by medical staff during abdominal examination. Swabs were placed in transport medium and inoculated on agar within six to 24 hours.

ISOLATION OF *S aureus*

Swabs were inoculated on to horse blood agar, incubated at 37°C for 24 hours. An aztreonam disc (30 µg; Oxoid) was placed on each inoculum to aid purification. *S aureus* was identified by colony morphology, pigment production, and slide agglutination techniques ("Staphaurex" rapid latex test kit; Wellcome). Dubious colonies were further subjected to tube coagulase testing. Purified isolates were phage typed at the Central Public Health Laboratory using the International Basic Set of typing phages at routine test dilution. Strains were stored in glycerol broth on glass beads at –70°C.

ANTIBIOTIC SENSITIVITY TESTING

Antibiotic sensitivity was tested by means of Oxoid "multo-disks" containing penicillin "G" 1.5 IU, tetracycline 10 µg, erythromycin 5 µg, clindamycin 2 µg, gentamicin 10 µg and neomycin 10 µg. Tests were performed at 37°C on Diagnostic Sensitivity Test Agar (Tissue Culture Services).

METHICILLIN SENSITIVITY TESTING

Mueller-Hinton agar was flooded with a heavy inoculum and incubated at 34–35°C for 24 and 48 hours. If the zone around the methicillin disc was less or equal to 13 mm the test was repeated using oxacillin 1 µg (OX1), methicillin 5 µg (MT5), and amoxycillin 2 µg (AML2) discs. Zone sizes were measured in the order OX1:MT5:AML2.

DETECTION OF EPIDERMOLYTIC ACTIVITY

Adult hairless mice were inoculated subcutaneously with about 10⁵ CFU staphylococci in 0.2 ml nutrient broth. A positive Nikolsky sign (wrinkling or peeling of the skin) was recorded two to four hours later in strains considered to have epidermolytic activity.^{13 17}

Results

GENERAL CARRIER RATE AND SITE OF CARRIAGE

It was found that 164 (33%) women carried *S aureus*; 100 (20%) carried strains in the nose, three (0.6%) in the axillae and 41 (8.2%) in the perineum (fig 1). Sixteen (3.2%) women carried *S aureus* in the nose and perineum, two (0.4%) in the nose and axillae, and in a further two (0.4%) carriage was evident in all three sites. More than one strain of *S aureus* was identified in seven of 20 women with multiple site carriage.

One of two subjects in whom *S aureus* was confirmed from all three sites produced the same strain (as shown by phage typing), but three different strains were obtained from the other. There were no cases in whom two or more different strains were isolated from the same anatomical site.

PHAGE TYPING

One hundred and eighty four strains were sent for phage typing and results were taken from reactions at routine test dilution. Sixty three (34%) strains were non-typable; 27 (15%) typed group I, 25 (14%) group II, and 36 (20%) group III (fig 2). Eight (4%) strains reacted with phage 95, and eight (4%) each typed groups I, III (95) and groups I and III. There were five (3%) group V reactions and two (1%) group I and II reactions. One isolate

Figure 1 Distribution of bacteriophage groups, MRSA, and epidermolytic toxin producing strains among *S aureus* isolates from antenatal women.

Figure 2 Proportion of staphylococcal carriers among 500 antenatal women and distribution of *S aureus* among nasal, axillary, and perineum carriage sites.

(0.5%) showed a group I and V combination and one (0.5%) reacted solely with phage 81. A single strain found in all three sites typed group II (55, 71) and three strains from one subject typed III (47, 53, 54, 84, 85), group I (79), and non-typable.

ANTIBIOTIC RESISTANCE PATTERNS

Antibiotic sensitivity testing showed that 57% of strains were resistant to penicillin; 25% were fully sensitive to all antibiotics tested; 10% strains were resistant to both penicillin and tetracycline, 3% resistant to penicillin and erythromycin, and 2% resistant to tetracycline alone. One strain was resistant to penicillin, gentamicin, and neomycin, one resistant solely to erythromycin, and one was resistant to penicillin, tetracycline, and neomycin.

METHICILLIN RESISTANCE AND EPIDERMOLYTIC TOXIN PRODUCTION

Three (less than 2%) strains were methicillin resistant; two were non-typable, and the third resembled EMRSA-2 typing group I (80). Five (3%) strains produced epidermolytic toxin as identified by the mouse bioassay. One typed group II (3A, 3C, 55, 71), three were non-typable, and the fifth typed group I and III (29, 52, 52A, 80, 47, 53, 75, 83A, 84).

Discussion

This survey was undertaken principally because of the occurrence of two outbreaks of an otherwise rare disease, the staphylococcal scalded skin syndrome, within a two year period.^{18 19} Speculation on the cause of these epidemics led us to question the incidence of epidermolytic toxin producing staphylococci in the community, because only hospital or clinical isolates have been screened for production of epidermolytic toxin.²⁰⁻²³

The overall carriage rate for *S aureus* (33%) is consistent with many reports on staphylococcal carriage.¹ The high perineal carriage rate (25% carriers), however, does not agree with previous findings — for example, Polakoff *et al* reported 13% perineal carriage among 361 anaesthetised patients,²⁷ and Solberg produced a similar result from 2014 patients screened on admission to hospital.²⁹ Black recorded rates of 1.9 and 2.1% for female and male perineal carriage, respectively.²⁵ The diverse results obtained from these and other studies may have been due in part to the difficulty in obtaining adequate perineal swabs from normal subjects. In this survey perineal swabbing of pregnant

women attending an antenatal clinic presented no difficulty because sampling was accepted as part of the normal routine and the mothers to be welcomed prior bacteriological screening.

More theoretical explanations for the higher rate of perineal carriage could, perhaps, be attributable to pregnancy: it has been reported that there is a dramatic rise in the number of staphylococci in the vaginal vestibule of sows 12 hours before giving birth.³⁰ It is also known from human studies of vaginal and vulval flora that shortly before delivery the vaginal flora consists mainly of coagulase negative staphylococci and there is therefore a predominance of these organisms on the skin of the newborn baby.³¹

When the results from 30 women newly admitted to the labour ward were examined it was found that nine (30%) carried *S aureus*, and in only one of these was carriage perineal. These women were included in the survey. If this high figure for perineal carriage is accepted nasal screening of a suspect population during an outbreak would miss one in four staphylococcal carriers. The observation that perineal carriage assists more readily in the dispersal of the organism also becomes more relevant.²⁹

The heightened metabolic rate seen in pregnancy, leading to increased activity of eccrine and apocrine glands, may also enhance staphylococcal carriage as *S aureus* is found principally in sites bearing these glands.¹ The incidence of axillary carriage was just 2%, however and overall percentage of carriage fell within normal limits (33%). Deodorant use mentioned by subjects at the time of swabbing probably did not affect carriage rate either.³²

Twenty (12%) staphylococcal carriers were multiple site carriers; of these, seven (4%) carried different strains of *S aureus*. Solberg found that more than one strain was carried by staphylococcal carriers on 10% of occasions.²⁹ Isolation of more than one strain may reflect sporadic or intermittent carriage before a resident strain becomes established.³³ No more than one strain from an anatomical site was found during this survey; this does not exclude coexistence of discrete minority populations, but it does support the finding that an avirulent strain may be planted in a carrier site to prevent colonisation by a potentially more pathogenic organism.³⁴

Phage typing results mirror the findings of a study by Ramamurti and Kanz, who reported that environmental strains from schools and sports facilities were 15% group I, 13% group II, 23% group III, and the rest, nearly half, non-typable.³⁵

Other reports differ in the proportions quoted — for example, group I 30%, group II 25%, group III 15% and the rest lysed by phages 42D, 81 or 187 or non-typable.¹ We were principally interested in the incidence of group II strains, because of their association with the staphylococcal scalded skin syndrome. Our results showed that pregnant women attending an antenatal clinic provided 14% group II strains, nearly twice the figures quoted from CPHL (8.2% and 7%) (Marple RR, personal communication).

The identification of methicillin resistant (MRSA) and epidermolytic toxin producing strains from the survey established the existence of a reservoir of potentially pathogenic staphylococci in the community especially as one MRSA strain resembled EMRSA-2.³⁶ Epidermolytic toxin producing strains were carried by five women out of the original group of 500, or 3% of 184 strains collected. If most adults have antibodies to epidermolytic toxins²⁴ then epidermolytic toxin producing strains must be readily transmissible among individuals in the community. The adult case we identified in the mini outbreak presumably had had no prior contact with a toxin producing strain.

Despite this knowledge our original question as to the cause of the outbreaks experienced in this hospital remains unanswered. There are probably several factors which contribute to the initiation and subsequent propagation of an epidemic. For example, individual policies and practices of the hospital concerned, domestic resources, ward layout, ventilation systems, age and architecture. It was long ago established that isolation measures could reduce the rate of postoperative wound infection,³⁷ and widespread environmental contamination, but not staff carriage, has been a feature in some outbreaks.³⁸ The susceptibility of a particular hospital population into which a strain is introduced is also relevant — in this case a maternity ward containing postpartum women and newborn babies; but adult cases of staphylococcal scalded skin syndrome, though very rare, are most often associated with immune suppression in, for example, renal patients.³⁹ Further points include personal habits of members of staff and the ability of a carrier to disperse an organism; staff have been implicated in two recent outbreaks in the United Kingdom^{18 40} and in other elsewhere. Finally there is the pathogenicity of the strain concerned.

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